

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
13 May 2004 (13.05.2004)

PCT

(10) International Publication Number
WO 2004/039255 A1

(51) International Patent Classification⁷: A61B 5/00, 6/00

(21) International Application Number:
PCT/GB2003/004672

(22) International Filing Date: 29 October 2003 (29.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0225060.3 29 October 2002 (29.10.2002) GB

(71) Applicant (for all designated States except US): ASTRON
CLINICA LIMITED [GB/GB]; The Mount, Toft, Cam-
bridge CB3 7RL (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE,
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT,
RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventor; and

(75) Inventor/Applicant (for US only): COTTON, Symon,
D'oily [GB/GB]; Rose Cottage, 42 Church Street, Great
Gransden, Cambridgeshire SG19 3AF (GB).

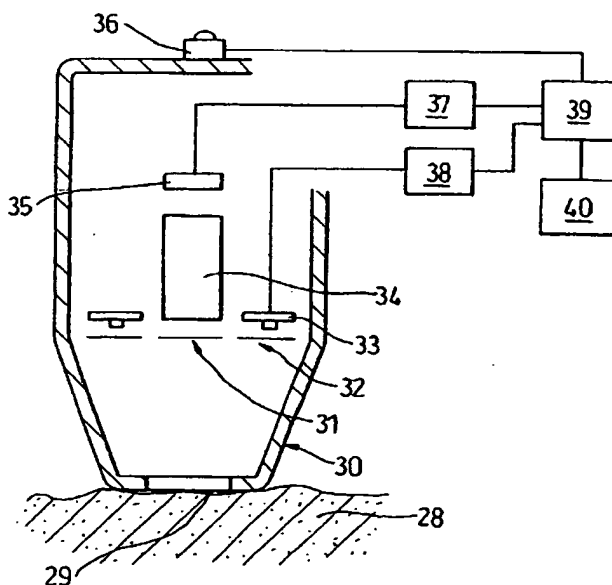
(74) Agent: BARKER BRETTELL; 138 Hagley Road, Edg-
baston, Birmingham B16 9PW (GB).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND APPARATUS FOR MEASURING TISSUE HISTOLOGY



(57) Abstract: A method of non-invasively analysing skin structure, comprising the steps of irradiating a plurality of locations over an area of tissue under investigation and detecting the light remitted at each location. An analysis of the light remitted at each location may then be made and a measurement representing the total melanin and dermal melanin within the tissue taken. Next a comparison of the measurement for the dermal melanin obtained previously is made with the measurement for the total melanin obtained previously. The comparison is then used to investigate the histology of the skin.

WO 2004/039255 A1

METHODS AND APPARATUS FOR MEASURING TISSUE HISTOLOGY

5 Field of the Invention

This invention relates to methods and apparatus for the non invasive measurement of epithelial tissue histology and is particularly, but not exclusively, concerned with measuring skin histology. These epithelial
10 tissues include the respiratory tract, the genital tract, the gastrointestinal tract and the retina of the eye. The present invention is considered to be potentially useful for the preliminary screening of patients to identify those who should be referred to an appropriate clinician for diagnosis and further to assist the clinician in diagnosis.

15

Background of the invention

The distribution of chromophores within these tissues and their lesions is considered to be potentially useful information for the screening of
20 patients to identify those who should be referred to an appropriate clinician for further diagnosis through tissue biopsy, or other conventional tests. Devices to detect these distributions with non invasive techniques are therefore beneficial. Further, the simplification of such devices and the automation of the diagnostic process will make such devices available
25 to wider populations.

The present invention improves the measurement technique described in WO 98/22023 and WO 00/75637. The devices described in this application are improvements of those described in PCT/GB01/01986 and
30 WO 02/02001.

Figure 11 shows a cross section of skin. The major structural layers are well known, cornified layer 45, epidermis 46, dermo-epidermal junction 47, papillary dermis 48, reticular dermis 49. In normal skin melanocytes 50, the cells responsible for the production of the pigment melanin, lie in the epidermis. In some benign skin lesions, such as the compound nevus melanocytes can exist in a characteristic pattern in both the epidermis and the papillary dermis. In melanoma the melanocytes can be asymmetrically distributed in both the epidermis and papillary dermis.

10 The present invention provides additional information on the distribution of the pigment melanin in the papillary dermis, and includes a method for analysing this data. This method is also applicable to other epithelial tissues with similar layered structures with differing optical properties.

15 Method to analyse presence and depth of chromophores in tissue

As can be seen from Figure 11, there are now four distinct layers within the dermis which can combine to construct a simple model, 1) a layer within the upper papillary dermis containing no melanin, 2) a layer within the upper papillary dermis containing melanin, 3) a layer within the lower
20 papillary dermis containing melanin, 4) a layer within the lower papillary dermis containing no melanin.

It should also be noted that the condition of melanin existing up to the dermo-epidermal junction is facilitated by allowing the thickness of layer 1 to be zero and likewise melanin can exist up to the papillary-reticular
25 dermis boundary by setting the thickness of layer 4 to be zero.

In computing a model to predict this coloration it is useful to make note of the fact that the amount of back scatter due to melanin can be considered negligible. Therefore, in the same manner that it was possible to apply the Kubelka-Munk theory to the papillary dermis to compute the

coloration of sections of papillary dermis containing blood, where the back scattering component of blood was considered negligible, it is possible to compute the coloration of sections containing melanin. In this situation $\zeta(\lambda)$ (scattering coefficient) remains dependent only on wavelength whilst α (fraction of radiation absorbed per unit path length) becomes $\alpha(\lambda, \rho, \phi)$ where ϕ represents the density of dermal melanin within that layer. Further $\alpha(\lambda, \rho, \phi)$ can be shown to be the sum of $\alpha_v(\lambda)$, $\alpha_b(\lambda)$ and $\alpha_m(\lambda)$, where $\alpha_m(\lambda)$ is the absorption coefficient of melanin. From the above it is possible to calculate R and T (diffuse radiation and transmission respectively). For simplicity of notation it is helpful to consider R1 and T1 where,

$$R_1(\lambda, \rho, \phi, d_n) = R(\beta(k(\alpha(\lambda, \rho, \phi)), s(\zeta(\lambda))), K(k(\alpha(\lambda, \rho, \phi)), s(\zeta(\lambda))), d_n)$$

and $T_1(\lambda, \rho, \phi, d_n) = T(\beta(k(\alpha(\lambda, \rho, \phi)), s(\zeta(\lambda))), K(k(\alpha(\lambda, \rho, \phi)), s(\zeta(\lambda))), d_n)$

where d_n is the layer thickness.

Two-layer systems can be combined to produce the total remitted and transmitted light for the dermis resulting in an equation which can be simplified using the geometric series

$$a + ar + ar^2 + ar^3 + \dots = \frac{a}{1-r} \text{ if } -1 < r < 1$$

to

$$R_{1total}(\lambda, \rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}) = R_{1ud}(\lambda, \rho_{ud}, d_{ud}) + \frac{T_{1ud}(\lambda, \rho_{ud}, d_{ud})^2 R_{1ld}(\lambda, \rho_{ld}, d_{ld})}{1 - R_{1ud}(\lambda, \rho_{ud}, d_{ud}) R_{1ld}(\lambda, \rho_{ld}, d_{ld})}$$

Similarly, T_{1total} can be shown to be

$$T_{1total}(\lambda, \rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}) = \frac{T_{1ud}(\lambda, \rho_{ud}, d_{ud}) \times T_{1ld}(\lambda, \rho_{ld}, d_{ld})}{1 - R_{1ud}(\lambda, \rho_{ud}, d_{ud}) R_{1ld}(\lambda, \rho_{ld}, d_{ld})}$$

These equations can be extended, as is shown by Wan et al. [1981], to an n layered system resulting in values for $R_{12\dots n}$ and $T_{12\dots n}$, of

$$R_{12\dots n} = R_{12\dots(n-1)} + \frac{T_{12\dots(n-1)}^2 R_n}{1 - R_{12\dots(n-1)} R_n}$$

4

$$T_{12...n} = \frac{T_{12...(n-1)} T_n}{1 - R_{12...(n-1)} R_n}$$

This system of equations can therefore compute the total remitted and transmitted light from an n layered system of arbitrary complexity provided that the thickness and composition of the layers is specified.

- 5 For the four-layer system shown in Figure 11, this results in a value for the total light remitted and transmitted from the dermis dependent on λ , ρ_{ud} , ρ_{ld} , d_{ud} , d_{ld} , d_{12} , ϕ_{12} , d_{13} and d_{13} where d_{12} and d_{13} are the thickness of layers 2 and 3 whilst ϕ_{12} and ϕ_{13} are their corresponding melanin densities. The thickness of layer 1 and layer 2 do not need to be explicitly defined
- 10 as they are simply $d_{ud} - d_{12}$ and $d_{ld} - d_{13}$ respectively; similarly ϕ_{11} and ϕ_{14} are zero by definition. A further simplification is possible if it is assumed that $\phi_{12} = \phi_{13}$ leading to a single value of ϕ for the dermis.

- The results of these equations can be combined with the predicted light transmitted by the epidermis in the same manner thus leading to the
- 15 following description of total remitted, S_{rd} , and transmitted S_{td} .

$$S_{rd}(\lambda, \rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi, d_m) = R_{2total}(\lambda, \rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi) \theta(\lambda, d_m)^2 S(\lambda)$$

$$S_{td}(\lambda, \rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi, d_m) = T_{2total}(\lambda, \rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi) \theta(\lambda, d_m)^2 S(\lambda).$$

These can be used to predict the value of the corresponding LMS primaries $L(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi, d_m) =$

20
$$\int_0^{\infty} R_{2total}(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi) \theta(\lambda, d_m)^2 S(\lambda) S_L(\lambda) d\lambda$$

$$M(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi, d_m) =$$

$$\int_0^{\infty} R_{2total}(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{12}, d_{13}, \phi) \theta(\lambda, d_m)^2 S(\lambda) S_M(\lambda) d\lambda$$

5

$$S(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi, d_m) = \int_0^{\infty} R_{2total}(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi) \theta(\lambda, d_m)^2 S(\lambda) S_s(\lambda) d\lambda$$

A further generalisation can be made to any primary, P_n , leading to the following equation where S_n defines the spectral response of that primary.

$$5 \quad P_n(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi, d_m) = \int_0^{\infty} R_{2total}(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi) \theta(\lambda, d_m)^2 S(\lambda) S_{P_n}(\lambda) d\lambda$$

(Equation 1)

This equation can then be used to generate the expected coloration of human skin exhibiting dermal descent of melanin.

10

This equation is more fully described in PCT publication WO 98/22023 and defines the expected colouration of human skin exhibiting the dermal descent of melanin.

15 Equation 1 may be modified to suit other tissues and chromophores and in the following description the terms 'skin' and 'melanin' represent a particular example application of this method. The technique is particularly applicable to the chromophore haemoglobin in the skin where this method can be readily applied.

20

Summary of the Invention

According to the invention there is provided a method of non-invasively analysing skin structure, comprising the steps of:

- (i) irradiating a plurality of locations over an area of tissue under investigation and detecting the light remitted at each location;
- (ii) analysing the light remitted at each location and obtaining a measurement representing the total melanin and dermal melanin within the tissue;
- (iii) comparing the measurement for the dermal melanin obtained in step (ii) with the measurement for the total melanin obtained in step (ii); and
- (iv) using the comparison obtained in step (iii) to investigate the skin.

An advantage of this method is that it facilitates the investigation of the skin histology and/or the condition of the skin.

15

This method is generally for analysing epithelial tissue histology and comprises irradiating a plurality of locations in an area of tissue under investigation with light and detecting light remitted at each of the plurality of locations to provide a spectral measurement over said plurality of locations.

20

In one embodiment the method comprises mapping each spectral measurement into a two dimensional colour space, the two primaries arranged such that variations in blood concentration in the tissue have substantially no effect in that space. In alternative, or additional embodiments more than two dimensions may be used. The skilled person will appreciate that the use of two dimensions compared to using further dimensions reduces the processing required to perform the method. The method may for example use 3, 4, 5, 6, 7, 8, 9, 10 or more dimensions.

30

The method may further comprise, for each spectral measurement calculating:

$$S(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi, d_m) = \int_0^{\infty} R_{total}(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi) \theta(\lambda, d_m)^2 S(\lambda) S_s(\lambda) d\lambda$$

5 where

d_{l2} and d_{l3} are the depths of the

d_{ud} and d_{ld} are the depths of the upper dermis and lower dermis respectively,

Φ is the density of melanin.

10

The method may further comprise plotting $S(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi, d_m)$ in that space to provide a series of contours of increasing dermal melanin concentration at the papillary dermis depth. The plotting of contours in this manner provides a convenient way of assessing the function.

15

In the example of measurement of the location of melanin in the skin this equation is plotted in Figure 1 with two primaries P1 & P2 chosen such that blood concentration is constant, and melanin can vary both in concentration and location in the skin.

20

Point 1 is the position of infinite melanin concentration in any position in the skin, point 2 is the point of non melanin in the skin. The line 5 connects points of increasing melanin concentration in the epidermis 45.

25

According to a second aspect of the invention there is provided a machine readable medium containing instructions which when read onto a machine cause that machine to perform the method of the first aspect of the invention.

The machine readable medium of any of the aspects of the invention may be any one or more of the following: a floppy disk; a CDROM/RAM; a DVD ROM /RAM (including +R/RW,-R/RW); any form of magneto
5 optical disk; a hard drive; a memory; a transmitted signal (including an internet download, file transfer, or the like); a wire; or any other form of medium.

There now follows by way of example only a detailed description of the
10 present invention with reference to the accompanying drawings in which

Figure 1 shows a graph of a function of dermal melanin depth, dermal melanin concentration and epidermal melanin concentration assuming a
15 constant dermal melanin concentration;

Figure 2 shows the graph of Figure 1 repeated for a range of dermal melanin concentrations;

Figure 3 shows a graph of a function of dermal melanin depth, dermal melanin concentration and epidermal melanin concentration assuming a
20 constant dermal melanin depth;

Figure 4 shows the graph of Figure 3 repeated for a range of dermal melanin depths;

25 Figure 5 shows a graph showing areas of similar value of a f_n (dermal melanin depth, dermal melanin concentration, epidermal melanin concentration);

30 Figure 6 shows a diagram of a typical skin lesion viewed from above;

Figure 7 to 9 show embodiments of devices that may be used for performing methods described herein;

5 Figure 10 shows a flowchart outlining steps in a method used in one embodiment of the invention;

Figure 11 shows a cross section of skin; and

10 Figure 12 shows a flowchart outlining steps in the methods described herein.

Figure 11 shows that there are three variables of interest that define the distribution of melanin in the skin. The concentration of melanin in the epidermis, the concentration of melanin in the papillary dermis and the location of melanin in the papillary dermis. Two simplified cases are considered, the first where the concentration of melanin in the papillary dermis is assumed to be uniform, and secondly that the depth of dermal melanin is either zero or the depth of the papillary dermis itself.

20 Standard dermal melanin concentration case

In Figure 1 point 3 is a point of chosen melanin concentration in the epidermis. As dermal melanin depth is increased (at standard concentration) the line 6 is generated. Point 4 represents the maximum possible depth that is the depth of the papillary dermis itself. This depth may have been standardised by the data calibration steps described in GB 9624003.1 but briefly which are as follows:

25 In one embodiment an image of the area of skin under investigation is represented in the same colour space as for the normal skin reference colour co-ordinate range. This can be done in a number of ways. In one way, the skin colour co-ordinates are acquired from an image using the same lighting conditions and a

CCD camera calibrated in the same way as that used to produce the healthy skin reference colour co-ordinate range. Alternatively, if exactly the same lighting conditions are not used, a white standard or other appropriate correction factor can be used to allow calibration of the image within the software. As a further
5 alternative, a colour image can be acquired using a colour photographic film which is then digitised. This can be performed using either exactly the same lighting conditions and a calibrated set-up or again with the inclusion of a white standard or other appropriate correction factor.

10 Figure 2 shows the effect of repeating line 6 with different amounts of dermal melanin. Lines 5,10,11,12 are then plotted joining points on these lines where the same dermal melanin depth is used. Lines 5,10,11,12 define areas 7,8,9 that represent areas where colours of shallow, medium and deep dermal melanin depth will lie.

15

Standard dermal melanin depth case

Figure 3 shows the same axes, curve 5 with end points 1 & 2 as described above. In this case a point of epidermal melanin concentration is chosen 13 and two lines
20 of low and high dermal melanin concentration are plotted with increasing depth 17, 16. The points at which the maximum dermal melanin depth is reached (papillary dermis depth) on these lines are 14 and 15.

Figure 4 shows these points 14, 15 joined to form lines of constant dermal
25 melanin concentration at maximum depth 18, 19,20. These lines bound areas 21,22,23 that represent areas where colours of low, medium and high dermal melanin concentration will lie, assuming dermal melanin exists and its maximum possible depth.

30 It can be seen from this analysis that a single point on these two axes is insufficient to fully define the dermal melanin depth, dermal melanin

concentration and epidermal melanin concentration. The following description outlines embodiments that overcome this problem and allow a clinically useful measurement to be made

- 5 Using actual values of melanin concentration in the epidermis, melanin concentration in the papillary dermis and dermal melanin depth that typically occur in human skin and in skin lesions, it was observed that areas 21, 22, 23 in Figure 4 and the areas 7, 8, 9 in Figure 2 were similar even though they were plotted using different simplification assumptions. These areas represent similar
- 10 values of a function of (dermal melanin depth, dermal melanin concentration, epidermal melanin concentration). This function can also be interpreted as an approximation to a measure of 'dermal melanin volume'. This approximation can be intuitively understood by considering Figure 4 and Figure 2. Line 5 represents tissue with no dermal melanin. Areas 21 (Figure 4) and 9 (Figure 2) represent tissue with
- 15 either deep, low concentration melanin, or shallow high concentration melanin (approximating to tissue with a low 'dermal melanin volume'). Whereas areas 23 (Figure 4) and 7 (Figure 2) represent tissue with deep, high concentrations of dermal melanin (approximating to tissue with high 'dermal melanin volume'). Line 20 represents the spectral response of tissue where significant melanin has
- 20 penetrated the papillary dermis. 'Dermal melanin volume' is clinically significant because in a typical melanoma lesion the melanin producing cells, melanocytes, are similar because they are all produced by reproduction from a single mutated cell, and therefore changes in melanin concentration are likely to be small compared to changes in the location of these cells. Therefore in such lesions areas
- 25 with increased 'dermal melanin volume' areas with increased dermal melanin depth. Dermal melanin depth is a well-known diagnostic and prognostic indicator in melanoma. If 'dermal melanin volume' is measured at an array of points across an area of skin, the measurements can be mapped to colours and displayed as a visualisation approximating to a map of dermal melanin depth across the lesion.
- 30 This can assist the clinician in determining the degree of chaos in the distribution of melanocytes within the lesion.

A particular technique to make measurements of f_n (dermal melanin depth, dermal melanin concentration, epidermal melanin concentration) or 'dermal melanin volume' is as follows. A spectral measurement is made using a device as described in Figures 7,8 and 9 or devices described in PCT/GB01/01986 or PCT/GB01/03011, and the measurements plotted on a graph indicating the areas of similar values of f_n (Dermal melanin depth, Dermal melanin concentration, Epidermal melanin concentration) as shown in Figure 5. This measurement can then be categorised by f_n (Dermal melanin depth, Dermal melanin concentration, Epidermal melanin concentration).

In one embodiment the method comprises mapping each spectral measurement into a two dimensional colour space, the two primaries arranged such that variations in blood concentration in the tissue have substantially no effect in that space. The mapping may be performed by projecting the a surface onto the two dimensional co-ordinate system. However, the skilled person will appreciate that other techniques are equally possible for mapping the measurement onto the two dimensional colour space.

A second particular technique to make measurements of f_n (dermal melanin depth, dermal melanin concentration, epidermal melanin concentration) or 'dermal melanin volume' is as follows. A spectral measurement is made using a device as described in Figures 7,8 & 9 or devices described in PCT/GB01/01986 or PCT/GB01/03011 and plotted on the graph shown in Figure 4. This measurement is compared to lines of changing dermal melanin concentration with constant dermal melanin depth. The line that intersects the measurement point provides a value of dermal melanin concentration which can be used as an approximation to the value of f_n (dermal melanin depth, dermal melanin concentration, epidermal melanin concentration). Figure 12 describes this process.

30

In one particular implementation of the process described in Figure 12 these

measurements are taken over a series of adjacent 15 micron square pixels over a 10 mm area, and the spectral measurements are made are the percentage remittance of light from the tissue at three separate primary wavebands with peaks at roughly 450nm 550nm and 650nm, each with a roughly 80nm
5 bandwidth. The skilled person will appreciate that in other embodiments other wavelengths for the peaks may be suitable. For example, peaks centered at roughly any of the following may be suitable: 400nm, 410nm, 420nm, 430 nm, 440 nm, 460nm, 470nm, 480nm, 490nm, 500nm, 510nm, 520nm, 530nm, 540nm, 560nm, 570nm, 580nm, 590nm, 600nm, 610nm, 620nm, 630nm,
10 640nm, 660nm, 670nm, 680nm, 690nm, 700nm or any value in between these values. Further, the bandwidth of the peak may be varied and may be roughly 50nm, 60nm, 70nm, 90nm, 100nm, 110nm, 120nm, 130nm, or any value between these values.

15 Alternatively the measured point could be plotted on Figure 2 where the measurement is compared to lines of changing dermal melanin depth with constant dermal melanin concentration. The line that intersects the point provides a value of dermal melanin depth which can be used as an approximation to the value of $fn(\text{dermal melanin depth, dermal melanin concentration, epidermal melanin concentration})$. In this case point 3 would represent a zero value, and
20 point 4 a maximum value.

If an array of spectral measurements are made and a corresponding array of $fn(\text{Dermal melanin depth, Dermal melanin concentration, Epidermal melanin concentration})$ is calculated, then a colour can be assigned to values of $fn(\text{dermal melanin depth, dermal melanin concentration, epidermal melanin concentration})$
25 and the data represented as a false colour image.

Further, the approximate value of $fn(\text{dermal melanin depth, dermal melanin concentration, epidermal melanin concentration})$ obtained from the above methods
30 can be further analysed to enable suspicious lesion to be differentiated from

benign ones. The following analysis technique can also be applied to arrays of other measurements made spatially across a tissue sample. In particular the measurements may be of blood or collagen concentration.

5 In the particular case of skin lesions, malignant skin lesions often contain the pigment melanin in the papillary dermis of the skin. A device that detects the presence of dermal melanin is effective at detecting malignant lesions and such devices are described in our patent applications GB0016690.0 and GB0112501.2. However these devices suffer from false positives generated by benign lesions
10 which also contain dermal melanin. It is well known that several types of benign skin lesions contain dermal melanin, including the compound nevus and blue nevus. Therefore to increase the specificity of a device to detect malignant lesions a method of differentiating benign lesions containing dermal melanin for malignant lesions is beneficial.

15

A well known property of malignant lesions is the asymmetric and chaotic growth of the lesion. Embodiments of this invention provide a method of automatically measuring the chaotic growth of the lesion.

20 Figure 6 shows a diagram of a typical skin lesion viewed from above. The skin 26 contains a melanocytic lesion 25, containing epidermal melanin as well as a region containing both epidermal and dermal melanin 27.

In one embodiment two data arrays are created to describe the lesion derived
25 from spectral measurements using the techniques described in the text above and our previous patents which are also detailed above and incorporated herein by reference.

In this particular embodiment one array contains values proportional to the total
30 amount of melanin in the skin, and a second with values approximating to $f_n(\text{dermal melanin depth, dermal melanin concentration, epidermal melanin}$

concentration). Where no dermal melanin exists this second array has zero values.

5 A well-known property of skin lesions is they have characteristic patterns in the distribution of melanin. Benign lesions have a variety of patterns, but these patterns are similar in both the epidermis and papillary dermis as melanocytes slowly descend from the dermal/epidermal junction into the papillary dermis. These melanocytes can also cease melanin production limiting dermal melanin to a shallow even layer in the papillary dermis. Malignant lesions characteristically
10 grow chaotically in three dimensions so the patterns of melanin distribution are significantly different in the epidermis and papillary dermis. This invention detects these differences through a mathematical process.

Figure 10 describes this process. In a particular implementation it operates on
15 two arrays of data generated from spectral measurements taken over an area of skin. One array is of measurements of total melanin calculated using the method described in GB9624003.1 and PCT/GB00/02124, and the second is an array of values of $f_n(\text{dermal melanin depth, dermal melanin concentration, epidermal melanin concentration})$ or 'dermal melanin volume' calculated from the process
20 described in Figure 12. The two arrays may provide images of the total melanin and the dermal melanin volume respectively.

In step 1 of Figure 10 the lesion is identified by applying a threshold to values of total melanin $TM(x,y)$. In step 2 the texture of the total melanin array within the
25 lesion is measured i.e. the apparent texture of the image represented by the array is determined. In one implementation the standard deviation SD is used to determine the texture, but other well known statistical measures could also be used such as range or fractal dimension. In order to determine the standard deviation the numbers within the array are taken as a series and the standard
30 deviation of these using known mathematical methods is taken.

In step 3 areas inside the lesion where dermal melanin is present are identified as those coordinates where the 'dermal melanin volume' $DMV(x,y)$ is not zero. In step 4 the same texture measurement is made of $DMV(x,y)$. In this implementation the standard deviation SD is used again in order to determine the texture of the image, but other well known statistical measures could also be used such as range or fractal dimension. In step 5 a ratio of the two texture measurements is made and in step 6 this is threshold is applied to determine malignancy. Calculating the ratio in this manner may be thought of as comparing the roughness of the texture of the images. It has been found that if the dermal melanin has a rougher texture than the 'dermal melanin volume' then the likelihood of a malignancy increases.

An improvement to this sequence is to add a preliminary step which detects elements in the array where hairs are present on the skin and marks them to be excluded from the calculation process. Erroneous data from the presence of hair has been found to disrupt this algorithm and provide unreliable results.

A particular embodiment of a device to implement the above techniques and those described in GB9624003, PCT/GB00/02124 and GB0112501.2 is described below and in Figures 7 and 8. This device consists of a light tight housing containing a transparent optical window 29 which is placed in contact with the skin 28. Light of a specific wavelength band is generated by light emitting diodes mounted on a ring 33. This ring may contain a number of groups of LED's each with it's own emission waveband. In one particular embodiment there are four groups of LED's, each group containing between 8 and 24 individual LED dies, this number depends on the brightness of the individual devices, where relatively dim devices are used in larger numbers to approximately equalise the illumination intensity across wave bands. In one particular embodiment the wavebands of each group of LED's span between 400nm and 1000nm. In one particular embodiment an additional reflector is mounted beside each LED to ensure a majority of the emitted light is reflected onto the window 19. In one particular embodiment an

additional filter is added over one or more of the LED groups to eliminate unwanted emissions in the infra-red region of the spectrum. This ring 33 is covered by a polarising filter 32. A feature of the design of the LED system is that the LED's are evenly spaced relative to the window 29 to ensure even illumination of the skin 28. The LED illuminator is controlled by an electronic driver system 38 which receives control signals from the processing means 39. The electronic driver system responds to signals received from the processing means and switches the LED groups on and off in sequence as required to synchronise with the camera system 35 and 37. In one embodiment the driver system can deliver different currents to each LED group, these currents can be generated according to data passed from the processing means 39 to the LED control system 38. In one embodiment the driver system can deliver different currents to each LED to compensate for the differences in efficiency of each device. Light remitted from the skin 28 passes through a second polarisation filter 31 mounted such that its polarisation axis is at 90 degrees relative to the polarisation axis of filter 32. This light then passes into the lens 34, and is focused onto a CCD sensor array 35. In a particular embodiment the lens 34 is designed to focus affectively over a wide waveband corresponding to the wavebands emitted by the LED illuminator 33. In a particular embodiment this waveband is from 400 nm to 1000 nm. The CCD sensor is controlled by an electronic system 37 which converts the light intensity on each array element into a digital pixel value in an image array which is passed to the processing means 39. In a particular implementation the processing means can control the exposure time and gain of the CCD array 35. The complete device is controlled by a control program implemented by the processing means 39. This program provides for a sequence of images to be acquired, each with a different illumination spectrum provided by the LED light source 33 and a corresponding exposure time. In one particular embodiment eight individual images are recorded, one with each of four wavelength bands with exposures set such that normal skin can be recorded. On the three wavelength bands which lie in the visible spectrum an additional over exposed image is recorded with 4 times the standard exposure

time for that wavelength band. The eighth image is taken with no illumination to determine the black values for each picture element. The image acquisition cycle is triggered by switch 36. The image data is then processed using algorithms described above. The process data is displayed on a screen 40.

5

One particular embodiment of this device is illustrated in Figure 8, in which items 29 to 38 are contained within a handset 41 and the processing means and display means are provided by a laptop computer 42. This provides a compact and portable implementation of this device.

10

In a second embodiment, illustrated in Figure 9, all items 29 — 40 are integrated into a hand held battery operated device. In this embodiment the output is simplified using the method described above and in Figure 10 and an output is presented a small LCD screen 43. The optical window 29 is shown as 44.

15

CLAIMS

1. A method of non-invasively analysing skin structure, comprising
5 the steps of:
 - (i) irradiating a plurality of locations over an area of tissue under investigation and detecting the light remitted at each location;
 - (ii) analysing the light remitted at each location and obtaining a
10 measurement representing the total melanin and dermal melanin within the tissue;
 - (iii) comparing the measurement for the dermal melanin obtained in step (ii) with the measurement for the total melanin obtained in step (ii); and
 - 15 (iv) using the comparison obtained in step (iii) to investigate the skin condition.
2. A method according to claim 1 where the dermal melanin measurement represents dermal melanin concentration.
20
3. A method according to claim 1 where the dermal melanin measurement represents dermal melanin depth.
4. A method according to claim 1 where the dermal melanin
25 measurement represents dermal melanin volume.
5. A method according to any preceding claim in which the dermal melanin measurement is shown as a false colour image.

6. A method according to any of any preceding claim in which an image is generated from the dermal melanin measurement made in step (ii).
- 5 7. A method according to claim 6 in which a texture measurement giving an indication of the texture of the image is calculated to give a measure of the total melanin.
8. A method according to claim 7 in which a standard deviation is
10 used to calculate the texture of the image and provide a measure of the total melanin from the image.
9. A method according to any preceding claim in which an image is generated from the total melanin measurement made in step (ii).
- 15 10. A method according to claim 9 in which a texture measurement giving an indication of the texture of the image is calculated to give a measure of the dermal melanin..
- 20 11. A method according to claim 10 in which a standard deviation is used to calculate the texture of the image and provide a measure of the dermal melanin from the image.
12. A method according to claim 10 as it depends from claim 7 in
25 which a comparison is made between the texture of the total melanin and the dermal melanin measurements.
13. A method according to claim 12 in which the comparison is made within regions where the dermal melanin is non-zero.

14. A method according to claim 12 or 13 where the comparison is made by utilizing a ratio of texture measurements.

5 15. A method according to any preceding claim which is used to infer information relating to skin malignancy.

16 A method according to any preceding claim in which step (ii) provides a spectral measurement over said plurality of locations.

10

17. A method according to claim 16 which comprises mapping each spectral measurement into at least a two dimensional colour space.

15 18. A method according to claim 17 in which the two dimensions of the two dimensional colour space are arranged such that variations in blood concentration in the tissue have substantially no effect in that space.

19. A method according to claim 17 or 18 which comprises, for each spectral measurement, calculating:

20
$$S(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi, d_m) =$$

$$\int_0^{\infty} R_{total}(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi) \theta(\lambda, d_m)^2 S(\lambda) S_s(\lambda) d\lambda$$

where

d_{l2} and d_{l3} are the depths of the

d_{ud} and d_{ld} are the depths of the upper dermis and lower dermis

25 respectively,

Φ is the density of melanin.

20. A method according to claim 21 which comprises plotting $S(\rho_{ud}, \rho_{ld}, d_{ud}, d_{ld}, d_{l2}, d_{l3}, \phi, d_m)$ in that space to provide a series of contours of increasing dermal melanin concentration at the papillary dermis depth.
- 5 21. A method according to claim 20 which comprises determining which contour is closest to the point represented by the spectral measurement; using that contour to provide an approximate value for fn (dermal melanin depth, dermal melanin concentration, epidermal concentration) for each location.
- 10 22. A method according to any of claims 16 to 21 in which the spectral measurements are calculated by calculating corrected colour co-ordinates for each location corresponding to a predetermined papillary dermis thickness.
- 15 23. A method according to any of claims 16 to 22 in which the spectral measurements are calibrated to eliminate effects due to variations in collagen concentration.
- 20 24. A method according to any claims 16 to 23, comprising the further step of making an array of fn values corresponding to each measurement location.
- 25 25. A method according to claim 24, for investigating a lesion upon tissue under investigation, comprising providing a second array of values of total melanin at each location, using said second array to measure the texture of the total melanin distribution (TTM); using said first array to measure the texture of the dermal melanin distribution (TDM), calculating the melanin texture ratio (MTR) for the lesion $MTR = TDM/TTM$.

26. A method according to claim 25, in which the MTR is compared with a threshold value to identify lesions with a high possibility of malignancy.

5 27. A method according to claim 25 or 26, in which the measurement of texture used for TTM is the same measure as used for TDM.

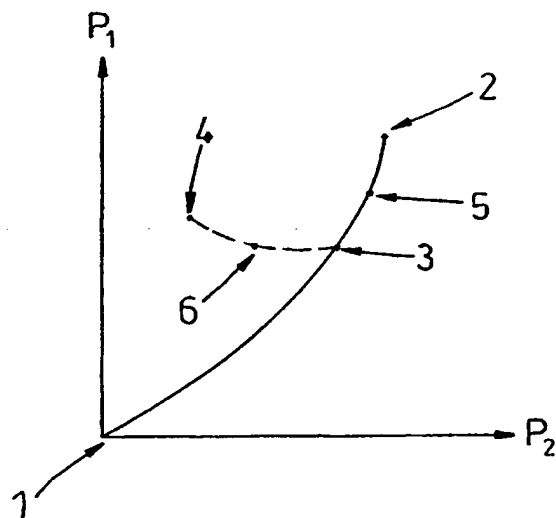
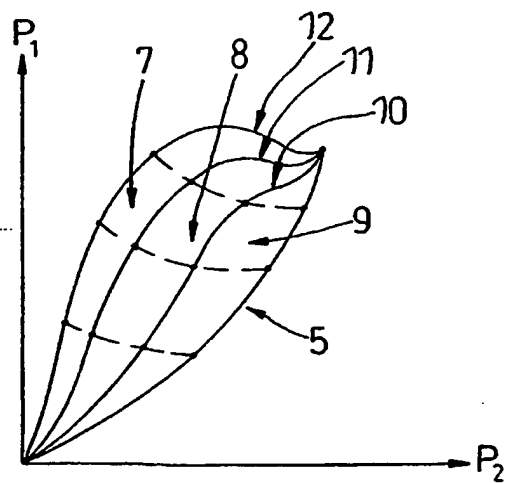
28. A method according to claim 27, in which the measurement of texture is by measuring the Standard deviation of the appropriate value.

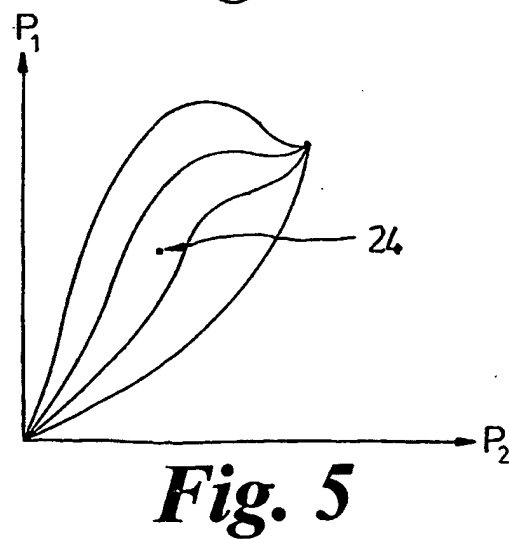
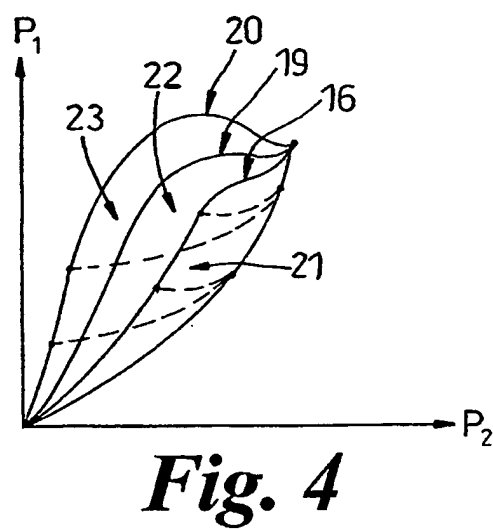
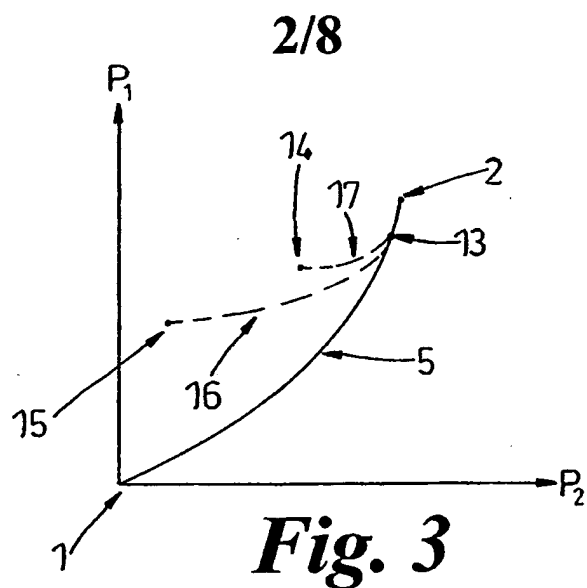
10

29. A machine readable medium containing instructions which when read onto a machine cause that machine to perform the method of any preceding claim.

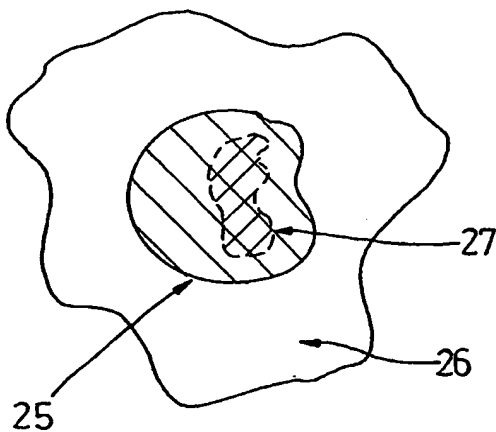
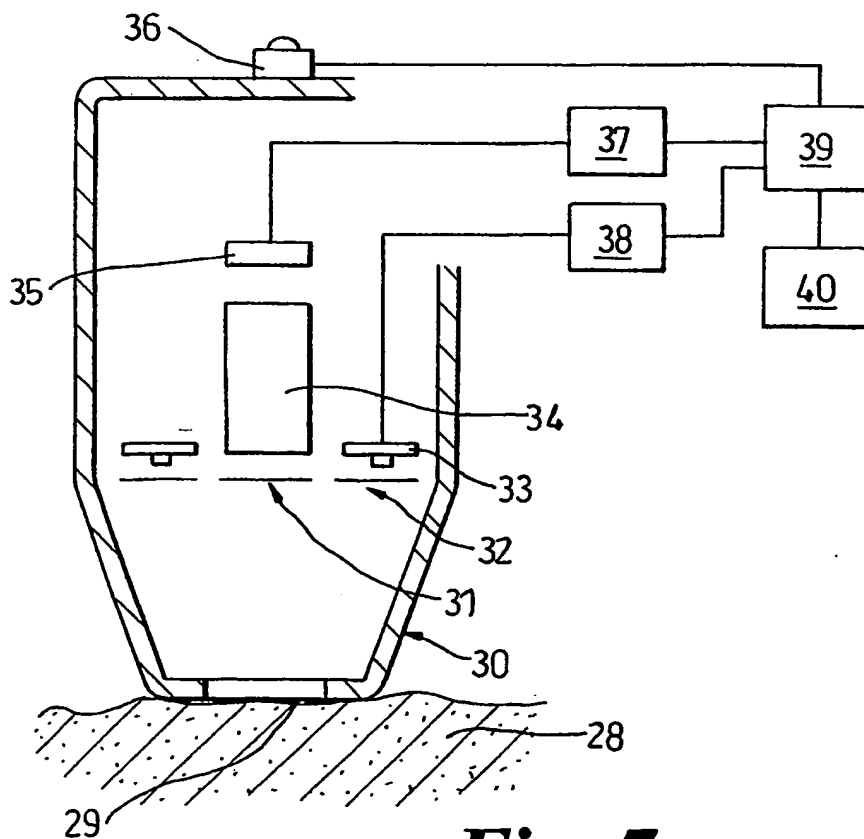
15 30. An apparatus arranged to non-invasively analyse the structure of skin comprising at least one light source arranged to irradiate the skin, a detector arranged to analyse light remitted from the skin to generate data representative of the remitted light and pass the data to a processor, the processor being arranged to process the data and obtain a measurement of
20 the dermal melanin and the total melanin in the skin from the data and further arranged to make a comparison between the dermal melanin and the total melanin in order to infer determine the histology of the skin.

1/8

**Fig. 1****Fig. 2**

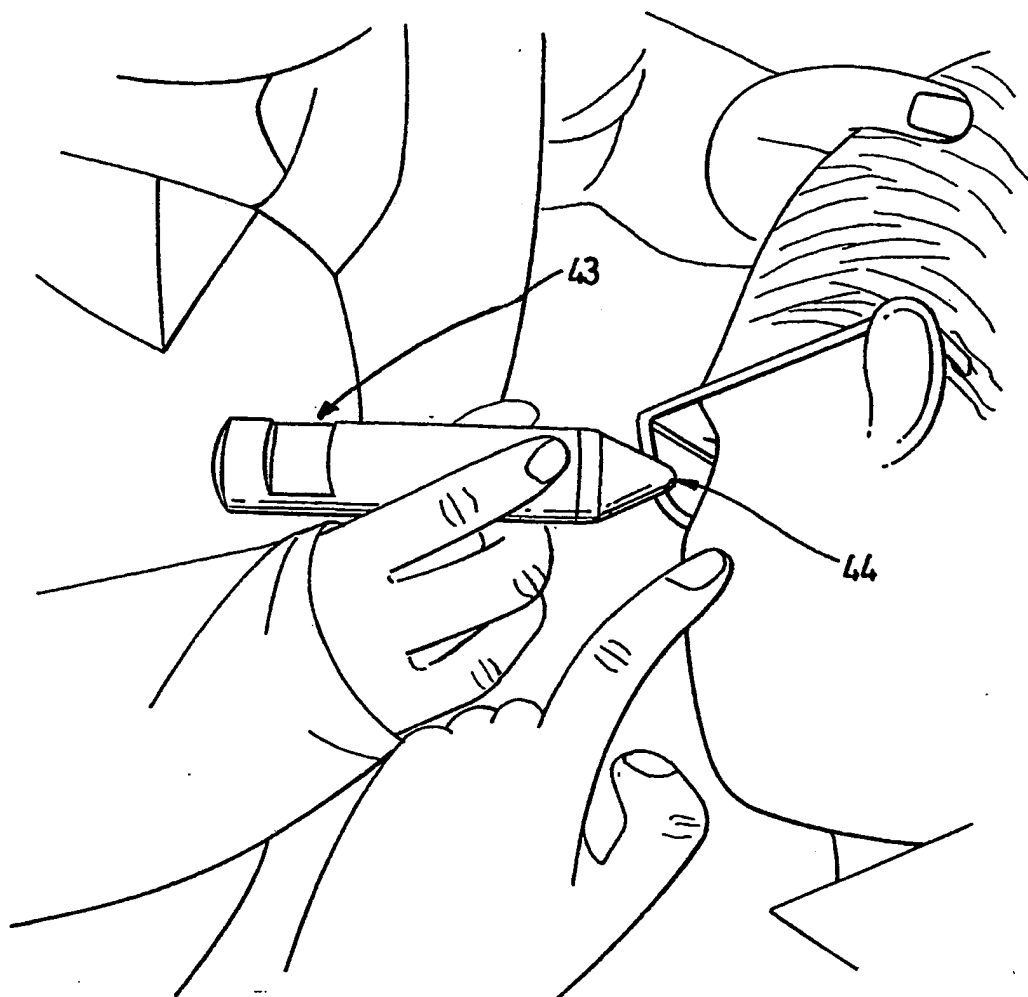


3/8

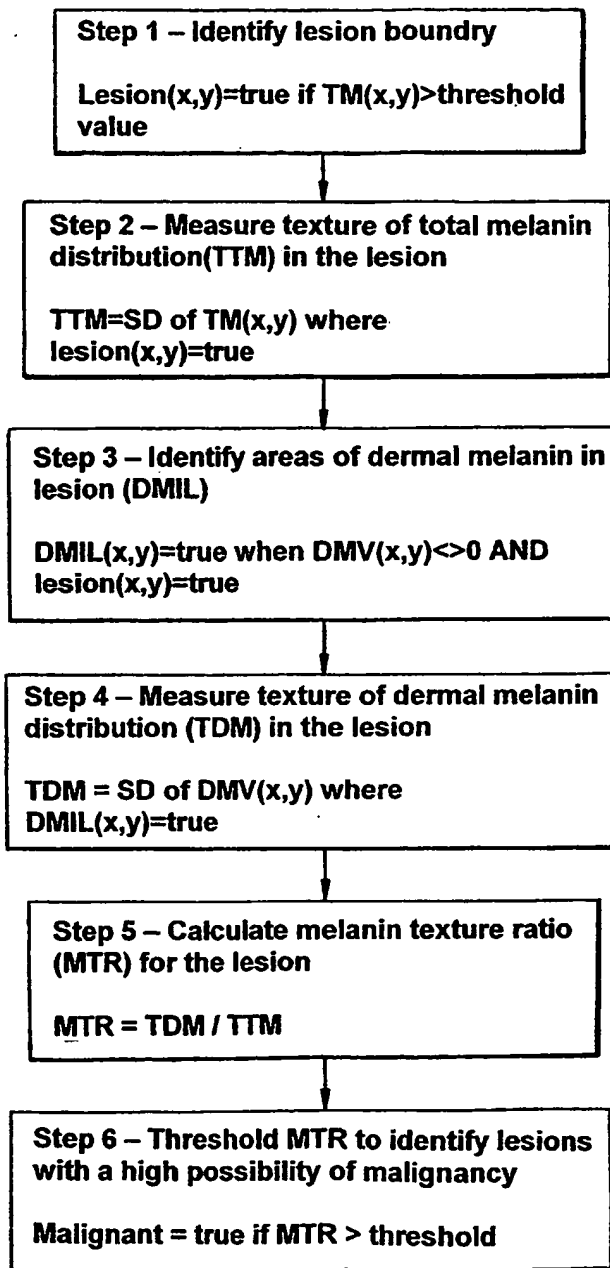
**Fig. 6****Fig. 7**

*Fig. 8*

5/8

**Fig. 9**

6/8

*Fig. 10*

7/8

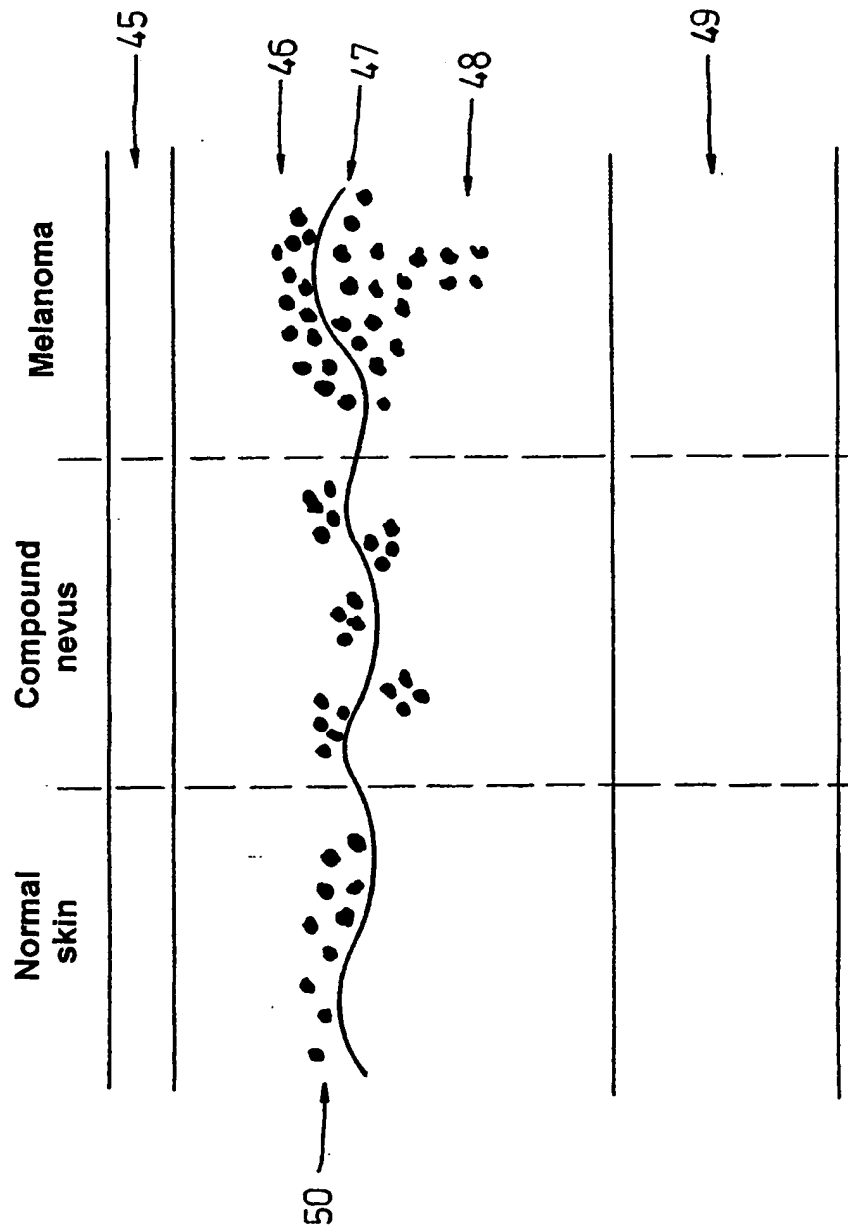
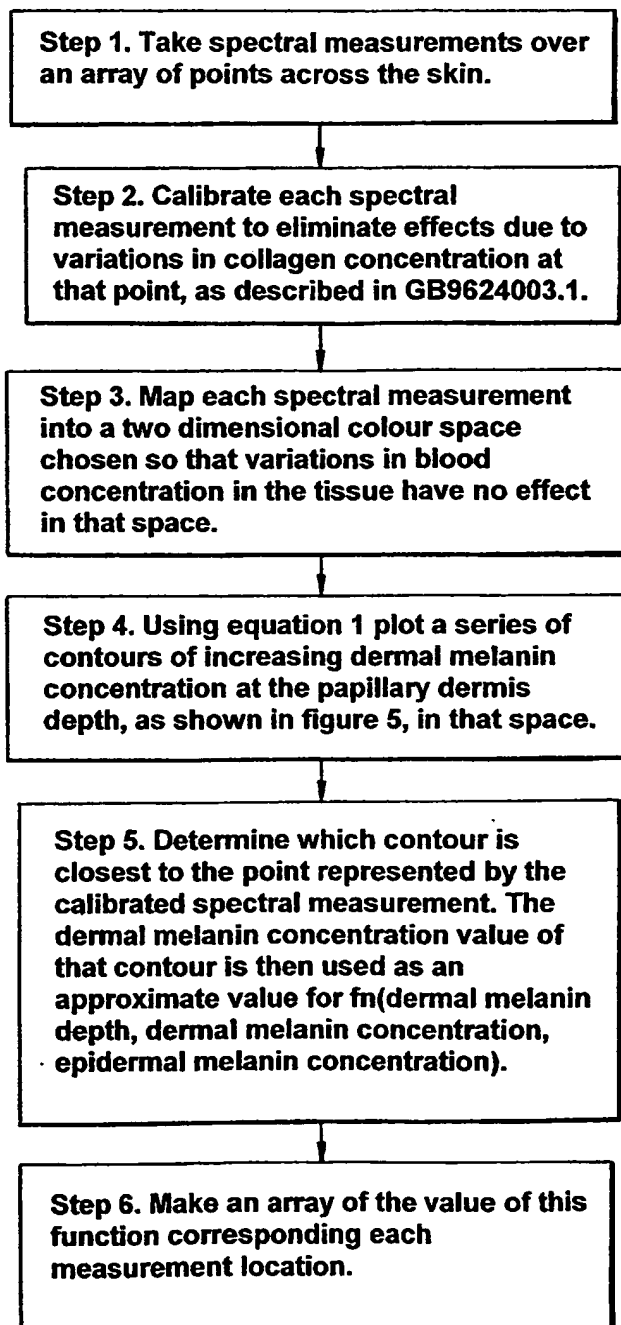
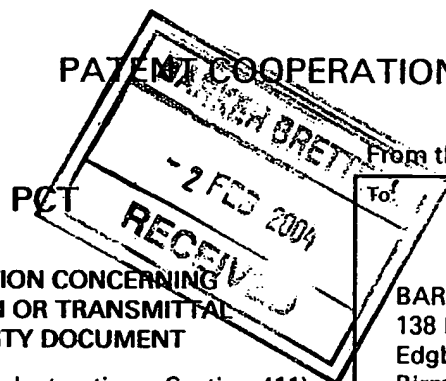


Fig. 11

8/8

*Fig. 12*

PATENT COOPERATION TREATY



From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

BARKER BRETTELL
138 Hagley Road
Edgbaston
Birmingham B16 9PW
United Kingdom

Date of mailing (day/month/year) 27 January 2004 (27.01.2004)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference JED 1223	
International application No. PCT/GB2003/004672	International filing date (day/month/year) 29 October 2003 (29.10.2003)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 29 October 2002 (29.10.2002)
Applicant ASTRON CLINICA LIMITED et al	

- By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
- (If applicable) The letters "NR" appearing in the right-hand column denote a **priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau** under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
- (If applicable) An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a **priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b)** (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
29 Octo 2002 (29.10.2002)	0225060.3	GB	20 Janu 2004 (20.01.2004)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338.89.65	Authorized officer PETRESKA Gorica Telephone No. (41-22) 338 9999
--	---